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THE PRIMARY QUANTUM CONVERSION PROCESS IN PHOTOSYNTHESIS: ELECTRON SPIN RESONANCE

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Department of Chemistry and Radiation Laboratory
University of California, Berkeley, California

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ABSTRACT

Photoinduced electron spin resonance signals have been observed in isolated chloroplasts and other green materials with a growth time not affected by reducing the temperature to -140°. This is interpreted in terms of conduction-band and trapped-electron theory.

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Some years ago--on the basis of a study of the kinetics of the Hill reaction in flashing light (1), together with a variety of independent chemical and kinetic studies of photosynthesis as well as the kinetics of some luminescence experiments (2)--a proposal was made concerning the nature of the early processes in the transformation of electromagnetic energy into chemical potential. This proposal involved an ordered array of chlorophyll and collateral molecules, suggested by the structural detail that has been provided for the photosynthetic organelles of green plants.

The sequence of events was proposed as an absorption leading to the first excited singlet state of chlorophyll, which then was converted into a triplet excitation, followed by an ionization process which would lead to a trapped electron and a hole; these two entities were the reducing and the oxidizing components that must be simultaneously generated. A part of the process involved the extremely rapid neutralization of the hole by capture of an electron from water, or from a product formed from it (3). The resultant material would then ultimately appear as molecular oxygen. The electron, on the other hand, would pass through a series of carriers (hydrogen carriers) such as are well known in biochemical processes (thioctic acid, pyridine nucleotide, flavin, etc.), ultimately leading to the reduction of carbon dioxide.

Although transient changes in the absorption spectrum of the photosynthetic particles during such a process have been observed, (5, 6), it has been difficult to identify the species responsible from such absorption changes in this complex of pigments. In such a sequence one might expect another physical property, more characteristic of at least two of these species, to be observable--namely, the magnetism of the triplet state of chlorophyll and the magnetism that would be associated with the trapped unpaired electrons.

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Although the direct observation of the changes in total magnetic susceptibility under illumination would be extremely difficult, it seemed likely to us that the sensitivity of the method of electron spin resonance might provide the means of making such a direct observation, and work toward this end was undertaken at that time. Early observations on various kinds of leaves and other plant materials gave indications that such an electron spin signal would appear under illumination, and in the middle of last year a report of the existence of such a light-induced signal in chloroplasts appeared (7). From the data there presented, as well as our own at that time, it was not possible to decide on the nature of the material responsible for the signal. Not only could it have been the chlorophyll triplet as proposed, or the trapped electron, but it could also have been radicals of the semiquinone type among the hydrogen carriers on the path between the trapped electron and carbon dioxide.

One possible way of eliminating secondary chemical reactions as a potential source of the signal is to reduce the temperature at which the illumination is carried out and determine the effect of this upon the rate at which the signal appears. A further variable that seemed to us important in the determination of the growth time of the signal is the rate at which quanta of light are actually impinging upon the sample. This we have also varied. And, finally, the nature of the green sample that is being examined may be expected to determine the growth and decay times of all of the various magnetic species we have mentioned. The electron spin resonance spectra of various samples under a variety of conditions were observed at a frequency of 9.3 kMc/sec (8), and the results are given in Table 1.

It is thus clear that the shortest growth times that we have so far observed are definitely limited by the rate at which quanta arrive at the sample. Secondly, it is clear that cooling to -140°, at which temperature we may expect none of the ordinary enzymatic reactions to proceed at measurable rates, does not appreciably lengthen the time required for the full signal to appear, while it does very materially lengthen the time required for the signal to disappear after the light is turned off. These facts are consistent with the idea that the signal is produced by the product of a physical process only, as distinct from an enzymatic or chemical one, as we normally conceive of it. The observation of a thermal luminescence and semiconductivity for dried chloroplasts has

recently been reported and interpreted as consistent with the idea that the chloroplastis have some of the properties of semiconductors (9).

The asymmetry of the signal itself (8), together with the fact that the decay--and probably the growth--is not a simple, single logarithmic one even at -140°, seems to call for more than one species as responsible for the over-all signal which we see. Among these species may be the chlorophyll triplet (as mentioned earlier), the trapped electron, and, finally, some species of free radical resulting from the direct dissociation of a chemical bond in the absorption act. While it is possible to suppose that the cooling would enhance the lifetime of the chlorophyll triplet to the extent of hours, it does not seem likely. We are thus left with the trapped electron and the possibilities of a dissociated bond.

It is perhaps worth noting that whatever the nature of the unpaired electron producing this signal, its coupling with the lattice around it must be rather poor in order to produce a signal as narrow as the one we see, suggesting its location in a rather delocalized pi-type of orbital. It is to be expected that improvements in technique will lead to a more precise identification of the variety of unpaired electrons which almost certainly result from the illumination of the photochemical apparatus in plants.

Table 1. Electron spin resonance observations on various samples from photosynthetic material.

Substance	Light intensity*	Temperature	Signal growth time	Signal decay time
Dried leaves	low	25°	minutes	hours
Dried whole chloroplasts (8)	low	25°	minutes	hours
		60°	seconds	seconds
Wet whole chloroplasts†	low	25°	seconds	minute
		-140°	seconds	hours
Wet small chloroplast fragments†	low	25°	seconds	hours
Wet large chloroplast fragments†	low	25°	~30 seconds	~30 seconds
	high	25°	~6 seconds	~30 seconds
	high	-140°	~10 seconds	hours

The g value of the resonance is 2.00.

At room temperature, the width between points of maximum slope $\cong$ 10 oersteds.

At -140°, the width between points of maximum slope $\cong$ 15 oersteds.

Light quality: $5800 < \lambda < 8000$ Å.

* Light intensity:

	Quanta/sec into cavity	Equilibrium number of free electrons
low:	$\sim 10^{15}$	$\sim 10^{16}$
high:	$\sim 10^{16}$	$\sim 10^{16}$

† N.G. Pon, thesis, University of California, Berkeley, to be filed June 1957. We are especially indebted to Mr. Pon for the preparation of these samples.

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